Amendments to the Specification

Please make the following amendments to the specification. The page and line numbers refer to the page and line in the published Application No. PCT/US2003/038684, published 17 June 2004.

At page 1, line 2, immediately following the title, insert the following new paragraph:

(New) This is the national phase application, under 35 U.S.C. § 371, for PCT/US2003/038684, filed December 4, 2003. This application claims the priority of U.S. Application No. 10/310,666, converted to U.S. Provisional Application No. 60/509,072, now abandoned.

Additional amendments are requested to correct numerous typographical, grammatical, and numbering errors in the specification, including the sequence listing. These amendments are first listed in table form, followed by a listing in paragraph form.

Page and line	Currently reads	Amend to read
p. 1, l. 28	paxlitaxel	paclitaxel
p. 2, l. 2	paxlitaxel	paclitaxel
p. 5, l. 15	cytotoxocity	cytotoxicity
p. 6, l. 3	SEQ ID NO: 202	SEQ ID NO: 200
p. 6, l. 4	SEQ ID NO: 201	SEQ ID NO: 199
p. 6, l. 7	SEQ ID NO: 204	SEQ ID NO: 201
p. 8, l. 6	buyrylcholinesterase	butyrylcholinesterase
p. 8, l. 10	variant the	variant having the
p. 8, l. 13	is	are
p. 8, l. 24	derviative	derivative
p. 9, l. 1	butyrylcolinesterase	butyrylcholinesterase
p. 10, l. 15	Denhart's	Denhardt's
p. 10, l. 18	Denhart's	Denhardt's
p. 10, l. 23	Ansubel	Ausubel
p. 11, l. 13	complimentary	complementary
p. 11, l. 28	butytlcholinesterase	butyrylcholinesterase
p. 13, l. 20	campthothecin	camptothecin
p. 14, l. 3	topoisonerases	topoisomerases
p. 14, l. 20	campthothecin	camptothecin
p. 14, l. 21	campthothecin	camptothecin
p. 14, l. 22	campthothecin	camptothecin
p. 28, l. 23	H2O	H_2O
p. 28, l. 28	occuring	occurring
p. 29, l. 2	occuring	occurring

Page and line	Currently reads	Amend to read
p. 29, l. 4	an D70G	a D70G
p. 29, l. 5	a E497V	an E497V
p. 30, l. 1	hinderance	hindrance
p. 33, l. 1	Imunother.	Immunother.
p. 34, l. 1	NOS: 198 and 200	NO: 198
p. 34, l. 5	butyrylcholoinesterase	butyrylcholinesterase
p. 34, l. 12	butyrylcholoinesterase	butyrylcholinesterase
p. 34, l. 13	202	200
p. 34, l. 14	201	199
p. 34, l. 16	202	200
p. 34, l. 18	204	201
p. 36, l. 12	butyrylcholiesterase	butyrylcholinesterase
p. 39, l. 3	occuring	occurring
p. 43, l. 8	Table 5	Table 3
p. 44, l. 29	Ansubel	Ausubel
p. 45, l. 4	activity activity	activity
p. 46, l. 1	desribed	described
p. 54, l. 6	paxlitaxel	paclitaxel
p. 54, l. 7 (two occurrences)	paxlitaxel	paclitaxel
p. 54, l. 14 (two occurrences)	paxlitaxel	paclitaxel
p. 54, l. 24	butyrylcholinesteras	butyrylcholinesterase
p. 55, l. 8	paxlitaxel	paclitaxel
p. 56, l. 26	202 or 204	200 or 201
p. 57, l. 8	202 or 204	200 or 201
p. 57, l. 23	Chemtoher	Chemother
p. 57, l. 29	NOS: 198 and 200	NO: 198
p. 58, l. 26	polypetides	polypeptides
p. 62, l. 22	synthesase	synthetase
p. 63, l. 21	phosphoramadites	phosphoramidites
p. 65, l. 11	was	were
p. 65, l. 13	Wittaker	Whittaker
p. 66, l. 15	100 ml/well	100 µL/well
p. 66, l. 16	250 ml/well	250 µL/well
p. 66, l. 18	200 ml	200 µL
p. 66, l. 20	250 ml/well	250 µL/well
p. 66, l. 21	85 ml/well	85 µL/well
p. 67, l. 1	15 ml	15 µL
p. 67, l. 26	Chromotography	Chromatography
p. 69, l. 8	Enchanced	Enhanced
p. 70, l. 7	tryptophane	tryptophan
p. 71, l. 12	200 ml	200 µL
p. 71, l. 14	250 ml/well	250 µL/well
p. 71, l. 15	250 ml/well	250 µL/well
p. 71, l. 16	200 ml	200 µL
p. 71, l. 18	180 ml	180 µL
p. 71, l. 19	Butyrythiocholine	Butyrylthiocholine

Page and line	Currently reads	Amend to read
p. 71, l. 20	20 ml	20 μL
p. 71, l. 24	results demonstrates	results demonstrate
p. 72, l. 5	50 ml/well	50 μL/well
p. 72, l. 7	250 ml/well	250 μL/well
p. 72, l. 8	250 ml/well	250 μL/well
p. 72, l. 9	200 ml	200 μL
p. 72, l. 11	180 ml	180 μL
p. 72, l. 12	Butyrythiocholine	Butyrylthiocholine
p. 72, l. 13	20 ml	20 μL
p. 72, l. 23	Piscatawy	Piscataway
p. 73, l. 6	butyrylcholinesterasae	butyrylcholinesterase
p. 73, l. 6	variants is	variants are
p. 73, l. 23	variants	variant
p. 74, l. 8	Figures 19 and 20	Figure 20
p. 74, l. 9	NOS: 198 and 200	NO: 198
p. 74, l. 9-10	NOS: 197 and 199	NO: 197
p. 74, l. 14	NO: 202	NO: 200
p. 74, l. 15	NO: 204	NO: 201
p. 75, l. 23	mg/m2	mg/m^2
p. 76, l. 5	identifation	identification

Shown below, in paragraph form, are the same amendments as shown above in table form:

- At page 1, line 24 through page 2, line 5, insert the following replacement paragraph:

Recently, classes of chemotherapeutic agents have been discovered which are activated within the body to produce a metabolic product which is toxic to cancer cells. These chemotherapeutic agents are sometimes referred to as "pro-drugs" since they are converted within the body to the active drug. Such chemotherapeutic agents include ~~paxlitaxel~~ **paclitaxel** prodrug and camptothecin (CPT-11). These agents are metabolized by endogenous carboxylesterases, such as butyrylcholinesterase, to yield active drugs such as ~~paxlitaxel~~ **paclitaxel** and SN-38, respectively. Unfortunately, although these chemotherapeutic agents have good antitumor activity *in vitro,* several side effects have been reported with these drugs in patients such as diarrhea, hair loss, nausea, vomiting, and cholinergic symptoms.

• At page 5, lines 15-16, insert the following replacement paragraph:

Figure 15 shows targeted ~~cytotoxocity~~ **cytotoxicity** against SKW tumor cells of an anti-CD20-butyrylcholinesterase fusion protein.

• At page 6, lines 3-9, insert the following replacement paragraph:

Figure 19 shows the amino acid sequence (SEQ ID NO: ~~202~~ **200**) and corresponding nucleotide sequence (SEQ ID NO: ~~201~~ **199**) of an anti-CD20 VH-CH1 hinge cys L530 BChE.4-1 heavy chain construct. This is the fusion protein heavy chain comprised of the anti-CD20 antibody variable heavy chain region with a cysteine-containing hinge region and the L530 fragment (SEQ ID NO: ~~204~~ **201**) of the butyrylcholinesterase variant designated SEQ ID NO: 180, which incorporates the 4-1 variant amino acids (H77F/F227A/P285N/V331A).

• At page 8, line 5 through page 9, line 23, insert the following two replacement paragraphs:

A butyrylcholinesterase variant can have a single amino acid alteration as well as multiple amino acid alterations compared to ~~buyrylcholinesterase~~ **butyrylcholinesterase**. A specific example of a butyrylcholinesterase variant is butyrylcholinesterase having the amino acid alanine at position 227, of which the amino acid sequence and encoding nucleic acid sequence is designated as SEQ ID NOS: 2 and 1, respectively. A further example is the butyrylcholinesterase ~~variant the~~ **variant having the** amino acid Phenylalanine at position 77, the amino acid alanine at position 227, the amino acid Asparagine at position 285, the amino acid alanine at position 331, of which the amino acid sequence and encoding nucleic acid sequence ~~is~~ **are** designated as SEQ ID NOS: 180 and 179, respectively, and which exhibits at least a three thousand-fold increased capability to convert the camptothecin derivative CPT-11 to the topoisomerase inhibitor SN-38 compared to butyrylcholinesterase. The term is also intended to include butyrylcholinesterase variants encompassing, for example, modified forms of naturally occurring amino acids such as D-stereoisomers, non-naturally occurring amino acids, amino acid analogues and mimetics so long as such variants have substantially the same amino acid sequence as butyrylcholinesterase and exhibit capability to convert a camptothecin derivative to a topoisomerase inhibitor. A butyrylcholinesterase variant of the invention can have one or amino acid alterations outside

of the regions determined or predicted to be important for conversion capability of a camptothecin ~~derviative~~ **derivative** to a topoisomerase inhibitor herein. Furthermore, a butyrylcholinesterase variant of the invention can have one or more additional modifications that do not significantly change its capability to convert a camptothecin derivative to a topoisomerase inhibitor activity. A butyrylcholinesterase variant of the invention can also have increased stability compared to butyrylcholinesterase.

As used herein, the ~~butyryleolinesterase~~ **butyrylcholinesterase** variants of the invention include sequences that are substantially the same as the reference amino acid sequence and a butyrylcholinesterase variant is thus intended to include a polypeptide, fragment or segment having an identical amino acid sequence, or a polypeptide, fragment or segment having a similar, non-identical sequence that is considered by those skilled in the art to be a functionally equivalent amino acid sequence. An amino acid sequence that is substantially identical to a reference butyrylcholinesterase or butyrylcholinesterase variant of the invention can have at least 70%, at least 80%, at least 81%, at least 83%, at least 85%, at least 90%, at least 95% or more identity to the reference butyrylcholinesterase. Substantially the same amino acid sequence is also intended to include polypeptides encompassing, for example, modified forms of naturally occurring amino acids such as D-stereoisomers, non-naturally occurring amino acids, amino acid analogues and mimetics so long as such polypeptides retain functional activity as defined above. A biological activity of a butyrylcholinesterase variant of the invention is the capability to convert a camptothecin derivative to a topoisomerase inhibitor, as described herein. For example, the butyrylcholinesterase variant F227A designated SEQ ID NO: 2 exhibits at least a three-fold increased capability to convert the camptothecin derivative CPT-11 to the topoisomerase inhibitor SN-38 compared to butyrylcholinesterase. A further example is the butyrylcholinesterase variant H77F, F227A, P285N, V331A designated SEQ ID NO: 180, which exhibits at least a three thousand-fold increased capability to convert the camptothecin derivative CPT-11 to the topoisomerase inhibitor SN-38 compared to butyrylcholinesterase.

• At page 10, lines 7-27, insert the following replacement paragraph:

The nucleic acid molecules of the invention include nucleic acid sequences that are substantially the same to the reference nucleic acid molecule of the invention or a fragment

thereof and are intended to include sequences having one or more additions, deletions or substitutions with respect to the reference sequence, so long as the nucleic acid molecule retains its ability to selectively hybridize with the subject nucleic acid molecule, under moderately stringent conditions, or highly stringent conditions. Moderately stringent conditions are intended to include hybridization conditions equivalent to hybridization of filter-bound nucleic acid in 50% formamide, 5 X ~~Denhart's~~ **Denhardt's** solution, 5 X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2 X SSPE, 0.2% SDS, at 50°. As used herein, highly stringent conditions are conditions equivalent to hybridization of filter-bound nucleic acid in 50% formamide, 5 X ~~Denhart's~~ **Denhardt's** solution, 5 X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2 X SSPE, 0.2% SDS, at 65°. Other suitable moderately stringent and highly stringent hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992) and in ~~Ansubel~~ **Ausubel** et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1998). Thus, it is not necessary that two nucleic acids exhibit sequence identity to be substantially complimentary, only that they can specifically hybridize or be made to specifically hybridize without detectible cross reactivity with other similar sequences.

• At page 11, line 8 through page 12, line 4, insert the following two replacement paragraphs:

As used herein, the term "fragment" when used in reference to a nucleic acid encoding the claimed polypeptides is intended to mean a nucleic acid having substantially the same sequence as a portion of a nucleic acid encoding a polypeptide of the invention or segments thereof. The nucleic acid fragment is sufficient in length and sequence to selectively hybridize to a butyrylcholinesterase variant encoding nucleic acid or a nucleotide sequence that is ~~complimentary~~ **complementary** to a butyrylcholinesterase variant encoding nucleic acid. Therefore, fragment is intended to include primers for sequencing and polymerase chain reaction (PCR) as well as probes for nucleic acid blot or solution hybridization.

Similarly, the term "functional fragment" when used in reference to a nucleic acid encoding a butyrylcholinesterase or butyrylcholinesterase variant is intended to refer to a

portion of the nucleic acid that encodes a portion of the butyrylcholinesterase or butyrylcholinesterase variant that still retains some or all of the metabolic conversion capability of the parent polypeptide. A functional fragment of a polypeptide of the invention exhibiting a functional activity can have, for example, at least 6 contiguous amino acid residues from the polypeptide, at least 8, 10, 15, 20, 30 or 40 amino acids, and often has at least 50, 75, 100, 200, 300, 400 or more amino acids of a polypeptide of the invention, up to the full length polypeptide minus one amino acid. An example of a functional fragment of a butyrylcholinesterase variant of the invention is a variant that is truncated at position 530, which is a Leucine residue in the wild-type ~~butytlcholinesterase~~ **butyrylcholinesterase**. While a L530 truncation has no effect on the functional activity of the corresponding full-length variant, the truncation prevents formation of tetramers, thereby enhancing bioactivity and pharmacokinetic properties of the variant. Therefore, a butyrylcholinesterase variant of the invention includes an L530 truncation, which is considered a functional fragment of the reference variant.

• At page 13, line 13 through page 14, line 6, insert the following two replacement paragraphs:

As used herein, the term "camptothecin derivative" refers to a compound that has a structure the same or substantially the same as camptothecin and that can be hydrolyzed by butyrylcholinesterase or a butyrylcholinesterase variant. For example, a camptothecin derivative can be hydrolyzed by the F227A/L286Q variant (SEQ ID NO:6). Camptothecin is derived from the stem bark of a Chinese tree called Camptotheca acuminata Decaisne. Camptothecin derivatives can inhibit DNA topoisomerase I through their metabolic break-down products. The structure of a water soluble ~~eampthothecin~~ **camptothecin** derivative, CPT-11, is shown in Figure 2. The chemical name of CPT-11 is 7-ethyl-10-[4-(1-piperidino)-1-piperidine]carbonyloxycamptothecin. CPT-11 is also known by the names CAMPTOSAR and Irinotecan. Members of the camptothecins include, for example, topotecan, irinotecan, 9-aminocamptothecin and 9-nitrocamptothecin which are analogs of the plant alkaloid 20(S)-camptothecin.

As used herein, the term "topoisomerase inhibitor" refers to a compound that can inhibit a topoisomerase. Several topoisomerases are known in the literature. For example, a

topoisomerase inhibitor can inhibit a type I topoisomerase, such as topoisomerase I, or a type II topoisomerase. Type I enzymes act by making a transient break in one strand of DNA and type II enzymes act by introducing a transient double strand break. Some DNA ~~topoisonerases~~ **topoisomerases** can relax or remove only negative supercoils from DNA while others can relax both negative and positive supercoils and still others can introduce negative supercoils. An example of a topoisomerase inhibitor is SN-38, the structure of which is shown in Figure 2.

• At page 14, lines 20-25, insert the following replacement paragraph:

As used herein, the term "~~campthothecin~~ **camptothecin** conversion activity" or ~~campthothecin~~ **camptothecin** hydrolysis activity is intended to mean the chemical conversion of a ~~campthothecin~~ **camptothecin** derivative to a topoisomerase inhibitor. For example, the conversion of CPT-11 to SN-38 is shown in Figure 2. Conversion activity can be measured both directly or indirectly using several assays described herein (see Example II, III, and IV).

• At page 28, line 17 through page 29, line 7, insert the following two replacement paragraphs:

Cholinesterases are ubiquitous, polymorphic carboxylase Type B enzymes capable of hydrolyzing the neurotransmitter acetylcholine and numerous ester-containing compounds. Two major cholinesterases are acetylcholinesterase and butyrylcholinesterase. Butyrylcholinesterase catalyzes the hydrolysis of a number of choline esters as shown:

BChE

Acetylcholine + ~~H20~~ **H_2O** -----> Choline + Corresponding Acid

Butyrylcholinesterase preferentially uses butyrylcholine and benzoylcholine as substrates. Butyrylcholinesterase is found in mammalian blood plasma, liver, pancreas, intestinal mucosa and the white matter of the central nervous system. The human gene encoding butyrylcholinesterase is located on chromosome 3 and over thirty naturally ~~occuring~~ **occurring** genetic variations of butyrylcholinesterase are known. The butyrylcholinesterase polypeptide is 574 amino acids in length and encoded by 1,722 base pairs of coding sequence. Three naturally ~~occuring~~ **occurring** butyrylcholinesterase variations are the

atypical alleles referred to as A variant, the J variant and the K variant. The A variant has ~~an D70G~~ **a D70G** mutation and is rare (0.5% allelic frequency), while the J variant has ~~a E497V~~ **an E497V** mutation and has only been found in one family. The K variant has a point mutation at nucleotide 1615, which results in an A539T mutation and has an allelic frequency of around 12% in Caucasians.

• At page 29, line 17 through page 30, line 8, insert the following replacement paragraph:

Naturally occurring human butyrylcholinesterase variations, species variations as well as recombinantly prepared mutations have previously been described by Xie et al., Molecular Pharmacology 55:83-91 (1999). A butyrylcholinesterase variant of the invention can be prepared by a variety of methods well known in the art. If desired, random mutagenesis can be performed to prepare a butyrylcholinesterase variant of the invention. Alternatively, as disclosed herein, random mutagenesis focused in discrete regions based on the information obtained from structural, biochemical and modeling methods described herein can be performed to target those amino acids predicted to be important for catalytic activity. For example, molecular modeling of a substrate in the active site of butyrylcholinesterase can be utilized to predict amino acid alterations that allow for higher catalytic efficiency based on a better fit between the enzyme and its substrate. In addition, molecular modeling can be used to predict amino acid alterations that decrease steric ~~hinderance~~ **hindrance** between the enzyme and substrate. Based on studies with cocaine as a substrate, residues predicted to be important for hydrolysis activity include 8 hydrophobic gorge residues and the catalytic triad residues. Furthermore, it is understood that amino acid alterations of residues important for the functional structure of a butyrylcholinesterase variant, which include the cysteine residues ^{65}Cys-^{92}Cys, ^{252}Cys-^{263}Cys, and ^{400}Cys-^{519}Cys involved in intrachain disulfide bonds are generally not altered in the preparation of a butyrylcholinesterase variant that has hydrolysis activity.

• At page 32, line 10 through page 33, line 2, insert the following replacement paragraph:

The invention further provides butyrylcholinesterase variants, or functional fragment thereof, that contains an antibody or antibody fragment. A butyrylcholinesterase variant of the invention can be fused to any antibody or antibody fragment. For example, a

butyrylcholinesterase variant of the invention can be fused to an antibody or antibody fragment that binds to a tumor-associated antigen. In this way the butyrylcholinesterase variant can be delivered directly to a tumor which can result in a decreased number of side effects. Several antigens are known to be over-expressed in tumor cells or expressed exclusively in tumor cells. These tumor associated antigens include, for example, Lewis Y (Siegall, C, Semin. Cancer Biol. 6:289-295 (1995)), carcinoembryonic antigen (CEA) (Watine et al., Dis. Colon Rectum 44:1791-1799 (2001)), tetraspanin L6 (Kaneko et al., Am. J. Gastroenterol. 96:3457-3458 (2001)), 17-lA (Indar et al., J.R. Coll. Surg. Edinb. 47:458-474 (2002)), mucin-1 (MUC-1) (Segal-Eiras and Croce, Allerg. Immunopath. 25:176-181 (1997)), epidermal growth factor receptor (EGFR) (Bookman, M., Semin. Oncol. 25:381-396 (1998)), cancer antigen 125 (CA 125) (Cherry and Vacchiano, Semin. Oncol. Nurs. 18:167-173 (2002)), p97 (Srivastava, P., Curr. Opin. Immunol. 3:654-658 (1991)), melanoma antigen gene (MAGE) (Barker and Salehi, J. Neurosci. Res. 67:705-712 (2002)), CD20 (Kosmas et al., Leukemia 16:2004-2015 (2002)), CD33 (Countouriotis et al., Stem Cells 20:215-229 (2002)), ganglioside GD2 (Ragupathi, G., Cancer Immunol. ~~Imunother~~. **Immunother.** 43:152-157 (1996)), and ganglioside GD3 (Ragupathi, G., *supra* (1996)).

• At page 33, line 22 through page 34, line 26, insert the following two replacement paragraphs:

In a further embodiment, the invention provides a butyrylcholinesterase variant where the antibody or antibody fragment specifically binds CD20. CD20 is a non-glycosylated phosphoprotein on the B-cell surface. CD20-antibody complexes do not internalize, thereby allowing cell-surface bound immunoglobulin to interact with effector cells or complement for a longer time. In one embodiment, the invention provides a butyrylcholinesterase variant where the antibody or antibody fragment contains an amino acid sequence as set forth in SEQ ID ~~NOS 198 and 200~~ **NO: 198**, and specifically binds CD20. CD20 is known to be up-regulated in several tumor cell types, for example, B cell lymphomas such as Non-Hodgkin's lymphoma as well in various autoimmune conditions.

Fusions between a butyrylcholinesterase variant and an antibody or antibody fragment can be used for targeted tumor cell-specific ~~butyrylcholoinesterase~~ **butyrylcholinesterase** mediated toxicity using a process called antibody-directed enzyme prodrug therapy (ADEPT)

(Jung, M., Mini Rev. Med. Chem. 1:399-407 (2001); Bagshawe, K.D., Mol. Med. Today 1:424-431 (1995); and Senter, P.D., FASEB J 4:188-193 (1990)). A related method called viral-directed enzyme prodrug therapy (VDEPT) can also be utilized. An example of a fusion between a butyrylcholinesterase variant and an antibody or antibody fragment that can be used for targeted tumor cell-specific ~~butyrylcholoinesterase~~ **butyrylcholinesterase** mediated toxicity is set forth as amino acid sequence designated SEQ ID NO: ~~202~~ **200** and corresponding nucleotide sequence set forth as SEQ ID NO: ~~201~~ **199**, which corresponds to an anti-CD20 VH-CH1 hinge cys L530 BChE.4-1 heavy chain construct. The butyrylcholinesterase variant designated SEQ ID NO: ~~202~~ **200** is a fusion protein that contains a heavy chain comprised of the anti-CD20 antibody variable heavy chain region with a cysteine-containing hinge region and the L530 functional fragment (SEQ ID NO: ~~204~~ **201**) of the butyrylcholinesterase variant designated SEQ ID NO: 180, which incorporates the 4-1 variant amino acids (H77F/F227A/P285N/V331A). VDEPT uses a viral vector to deliver an enzyme such as a butyrylcholinesterase variant of the invention. With such approaches, selective expression of an enzyme can efficiently activate non-toxic or moderately toxic prodrugs in tumor cells into highly toxic metabolic products resulting in enhanced anti-tumor activity and an improved therapeutic index. In order for these approaches to be successful the enzyme needs to be of high activity, for example, the butyrylcholinesterase variants of the invention can be used.

- At page 36, lines 9-18, insert the following replacement paragraph:

In addition to structural modeling of butyrylcholinesterase, biochemical data can be used to determine or predict regions of butyrylcholinesterase important for camptothecin conversion or hydrolysis activity when preparing a focused library of ~~butyrylcholiesterase~~ **butyrylcholinesterase** variants. In this regard, the characterization of naturally occurring butyrylcholinesterase variants with altered camptothecin conversion or hydrolysis activity is useful for identifying regions important for the catalytic activity of butyrylcholinesterase. Similarly, site-directed mutagenesis studies can provide data regarding catalytically important amino acid residues as reviewed, for example, in Schwartz et al., Pharmac. Ther. 67: 283-322 (1992), which is incorporated by reference.

• At page 38, line 21 through page 39, line 6, insert the following replacement paragraph:

The generation of a library of nucleic acids encoding butyrylcholinesterase variants can be by any means desired by the user. Those skilled in the art will know what methods can be used to generate libraries of nucleic acids encoding butyrylcholinesterase variants. For example, butyrylcholinesterase variants can be generated by mutagenesis of nucleic acids encoding butyrylcholinesterase using methods well known to those skilled in the art (Molecular Cloning: A Laboratory Manual, Sambrook et al., eds., Cold Spring Harbor Press, Plainview, NY (1989)). A library of nucleic acids encoding butyrylcholinesterase variants of the invention can be randomized to be sufficiently diverse to contain nucleic acids encoding every possible naturally occurring amino acid at each amino acid position of butyrylcholinesterase. Alternatively, a library of nucleic acids can be prepared such that it contains nucleic acids encoding every possible naturally ~~occuring~~ **occurring** amino acid at each amino acid only at positions located within a region of butyrylcholinesterase predicted or determined to be important for camptothecin conversion or hydrolysis activity.

• At page 43, lines 1-8, insert the following replacement paragraph:

The invention library of nucleic acids encoding butyrylcholinesterase variants can be expressed in a variety of eukaryotic cells. For example, the nucleic acids can be expressed in mammalian cells, insect cells, plant cells, and non-yeast fungal cells. Mammalian cell lines useful for expressing the invention library of nucleic acids encoding butyrylcholinesterase variants include, for example, Chinese Hamster Ovary (CHO), human 293T and Human NIH 3T3 cell lines. Expression of the invention library of nucleic acids encoding butyrylcholinesterase variants can be achieved by both stable or transient cell transfection (see Example III, ~~Table 5~~ **Table 3**).

• At page 44, line 25 through page 45, line 8, insert the following replacement paragraph:

Following the expression of a library of butyrylcholinesterase variants in a mammalian cell line, randomly selected clones can be sequenced and screened for increased catalytic activity. Methods for sequencing selected clones are well known to those of skill in the art and are described, for example, in Sambrook et al., supra, 1992, and in ~~Ansubel~~ **Ausubel** et al., supra, 1998. Selecting a suitable, method for measuring the camptothecin

conversion or hydrolysis activity of a butyrylcholinesterase variant depends on a variety of factors such as, for example, the amount of the butyrylcholinesterase variant that is available. The camptothecin conversion or hydrolysis ~~activity activity~~ **activity** of a butyrylcholinesterase variant can be measured, for example, by spectrophotometry, by a microtiter-based assay utilizing a polyclonal anti-butyrylcholinesterase antibody to uniformly capture the butyrylcholinesterase variants and by high-performance liquid chromatography (HPLC).

• At page 45, line 19 through page 46, line 5, insert the following replacement paragraph:

Clones expressing a butyrylcholinesterase variant with increased camptothecin hydrolysis activity can be used to established larger-scale cultures suitable for purifying larger quantities of the butyrylcholinesterase. A butyrylcholinesterase variant of interest can be cloned into an expression vector and used to transfect a cell line, which can subsequently be expanded. Those skilled in the art will know what type of expression vector is suitable for a particular application. A butyrylcholinesterase variant exhibiting increased camptothecin conversion or hydrolysis activity can be cloned, for example, into an expression vector carrying a gene that confers resistance to a particular chemical agent to allow positive selection of the transfected cells. An expression vector suitable for transfection of, for example, mammalian cell lines can contain a promoter such as the cytomegalovirus (CMV) promoter for selection in mammalian cells. As ~~desribed~~ **described** herein, a butyrylcholinesterase variant can be cloned into a mammalian expression vector and transiently transfected into human 293T cells. Expression vectors suitable for expressing a butyrylcholinesterase variant are well known in the art and commercially available.

• At page 54, line 6 through page 55, line 13, insert the following two replacement paragraphs:

The invention also provides a method of converting a ~~paxlitaxel~~ **paclitaxel** prodrug to a ~~paxlitaxel~~ **paclitaxel** by contacting the ~~paxlitaxel~~ **paclitaxel** prodrug with a butyrylcholinesterase variant selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120,

122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, and 196, or functional fragment thereof, under conditions that allow conversion of a ~~paxlitaxel~~ **paclitaxel** prodrug to ~~paxlitaxel~~ **paclitaxel**. In one embodiment, the butyrylcholinesterase variant exhibits a two-fold or greater increase in conversion capability compared to butyrylcholinesterase, a ten-fold or greater increase in conversion capability compared to butyrylcholinesterase, a fifty-fold or more enhanced conversion capability compared to butyrylcholinesterase, a one hundred-fold or more enhanced conversion capability compared to butyrylcholinesterase, a two hundred-fold or more enhanced conversion capability compared to butyrylcholinesterase, a three hundred-fold or more enhanced conversion capability compared to butyrylcholinesterase, a four hundred-fold or more enhanced conversion capability compared to butyrylcholinesterase, a five hundred-fold or more enhanced conversion capability compared to ~~butyrylcholinesteras~~ **butyrylcholinesterase**, a one thousand-fold or more enhanced conversion capability compared to butyrylcholinesterase, a fifteen hundred-fold or more enhanced conversion capability compared to butyrylcholinesterase, a two thousand-fold or more enhanced conversion capability compared to butyrylcholinesterase, a three thousand-fold or more enhanced conversion capability compared to butyrylcholinesterase.

Paclitaxel prodrugs such as paclitaxel-2-ethylcarbonate (PC) have significant levels of antitumor activities in rodent models of human cancers. Paclitaxel (also known as TAXOL) was originally isolated from the bark of the Pacific yew tree and has been used in the treatment of several cancers including, for example, breast cancer, ovarian cancer, non-small cell lung cancer and Kaposi's sarcoma. The mechanism of action of this class of chemotherapeutic agents is the stabilization of tubulin. Serum carboxylesterases such as rat carboxylesterase has been shown to convert paclitaxel prodrugs, such as PC, to ~~paxlitaxel~~ **paclitaxel**. These serum carboxylesterases enhance the cytotoxic activity of PC on lung carcinoma and melanoma cell lines (Senter et al., Cancer Res. 56:1471-1474 (1996)). Butyrylcholinesterase and butyrylcholinesterase variants of the invention can be used to convert paclitaxel prodrugs such as PC into active drugs useful for the treatment of cancer.

• At page 56, line 19 through page 58, line 11, insert the following four replacement paragraphs:

The invention also provides a method of treating cancer by administering to an individual an effective amount of a butyrylcholinesterase variant selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, ~~202 or 204~~ **200 or 201** or functional fragment thereof, exhibiting increased capability to convert CPT-11 to a topoisomerase inhibitor compared to butyrylcholinesterase. In one embodiment, the topoisomerase inhibitor is SN-38.

The invention further provides a method of treating cancer by administering to an individual an effective amount of a butyrylcholinesterase variant selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, ~~202 or 204~~ **200 or 201**, or functional fragment thereof, exhibiting increased capability to convert a paclitaxel prodrug to paclitaxel compared to butyrylcholinesterase. In one embodiment, the cancer is metastatic colorectal cancer. In another embodiment the cancer is ovarian cancer. In another embodiment the cancer is breast cancer. In a further embodiment the cancer is lung cancer. In a still further embodiment the cancer is Kaposi's sarcoma.

Paclitaxel and camptothecin derivatives are known to be effective chemotherapeutic agents against a variety of cancers. For example, CPT-11 has been approved by the FDA for the treatment of colon cancer. Improvements in the hydrolysis of CPT-11 to SN-38 will aid in the usefulness of this drug and reduce side-effects in patients. For example, side-effects of CPT-11 treatment can include diarrhea, hair loss, nausea, vomiting, myelosuppression, hyperglycemia, alopecia and cholinergic symptoms (Moertel et al., Cancer Chemo. R. 56:95-101 (1972); Muggia et al., Ca. Chemother. Rep. 56:515-521 (1972)). In addition to colon

cancer, these drugs have been tested in a variety of other cancers (see Hare et al., Cancer ~~Chemtoher.~~ **Chemother**. Pharmacol. 39:187-191 (1997), incorporated herein by reference).

The invention provides a method of treating cancer in an individual by administering a therapeutically effective amount of the butyrylcholinesterase variant. It is contemplated that a method of treating cancer in an individual by administering a therapeutically effective amount of the butyrylcholinesterase variant can be administration of a variant that further contains an antibody or antibody fragment, for example, the CD20 (SEQ ID ~~NOS: 198 and 200~~ **NO: 198**) and EGF (SEQ ID NOS: 18 and 20) antibodies and corresponding fragments described herein. The dosage of a butyrylcholinesterase variant required to be effective depends, for example, on the route and form of administration, the potency and bio-active half-life of the molecule being administered, the weight and condition of the individual, and previous or concurrent therapies. The appropriate amount considered to be an effective dose for a particular application of the method can be determined by those skilled in the art, using the teachings and guidance provided herein. For example, the amount can be extrapolated from *in vitro* or *in vivo* butyrylcholinesterase assays described herein. One skilled in the art will recognize that the condition of the individual needs to be monitored throughout the course of treatment and that the amount of the composition that is administered can be adjusted accordingly.

• At page 58, line 24 through page 59, line 7, insert the following replacement paragraph:

A butyrylcholinesterase variant can be delivered systemically, such as intravenously or intraarterially. A butyrylcholinesterase variant can be provided in the form of isolated and substantially purified ~~polypetides~~ **polypeptides** and polypeptide fragments, in pharmaceutically acceptable formulations using formulation methods known to those of ordinary skill in the art. These formulations can be administered by standard routes, including for example, topical, transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, oral, rectal or parenteral (e, g., intravenous, intraspinal, subcutaneous or intramuscular) routes. In addition, a butyrylcholinesterase variant can be incorporated into biodegradable polymers allowing for sustained release of the compound useful for treating individual symptomatic of cancer.

Biodegradable polymers and their use are described, for example, in detail in Brem et al., J. Neurosurg. 74:441-446 (1991), which is incorporated herein by reference.

• At page 62, lines 19-24, insert the following replacement paragraph:

The 1.8-kb fragments constituting the butyrylcholinesterase variants were cloned into the plasmid pGS and resequenced to make sure the desired mutation was present. The plasmid pGS is identical with pRc/CMV (Invitrogen, Carlsbad, CA) except that the Neo gene has been replaced by rat glutamine ~~synthesase~~ **synthetase**. These variants can be stably expressed, for example, in Chinese Hamster Ovary (CHO) cell lines, or transiently expressed, for example, as described below in 293T cells.

• At page 63, lines 14-23, insert the following replacement paragraph:

Libraries of nucleic acids corresponding to the seven regions of human butyrylcholinesterase to be mutated are synthesized by codon-based mutagenesis, as described above. Briefly, multiple DNA synthesis columns are used for synthesizing the oligonucleotides by ß-cyanoethyl phosphoramidite chemistry, as described previously by Glaser et al., supra 1992. In the first step, trinucleotides encoding for the amino acids of butyrylcholinesterase are synthesized on one column while a second column is used to synthesize the trinucleotide NN(G/T), where N is a mixture of dA, dG, dC, and dT cyanoethyl ~~phosphoramadites~~ **phosphoramidites**. Using the trinucleotide NN(G/T) results in thorough mutagenesis with minimal degeneracy, accomplished through the systematic expression of all twenty amino acids at every position.

• At page 65, lines 3-17, insert the following replacement paragraph:

Butyrylcholinesterase variants that contain double mutations were expressed in a transient system using 293T human embryonic kidney cells. Briefly, on day 1, 293T cells were plated at 1.5 x 10^5 cells/well in a BioCoat 24-well plate. The cells were then allowed to recover overnight. On the second day, dilute 2µl of Lipofectamine 2000/well in 50µl Opti-MEM/well, and incubate 5 minutes. Dilute 500ng-1µg DNA/well in 50µl Opti-MEM/well. The two diluted solutions were mixed, and incubated for 20 minutes at room temperature.

Media was removed from cells and replaced with 500µl/well complete growth media, without penicillin or streptomycin. Subsequently, 100µl of diluted solutions ~~was~~ **were** added to each well, and incubated on cells for 4 hours. The media/DNA/Lipofectamine 2000 was removed from cells, and replaced with 1 ml of Ultraculture serum free media (Bio ~~Wittaker~~ **Whittaker**) per well. The butyrylcholinesterase variant polypeptides were allowed to accumulate for 48-96 hours and the conditioned media from the cells was used directly. For other applications the butyrylcholinesterase variant polypeptides can be purified as described below in Example VI.

• At page 66, line 15 through page 67, line 2, insert the following replacement paragraph:

Protocol to Determine Carboxylesterase activity of Captured Butyrylcholinesterase by o-Nitrophenyl acetate:

1) Coat 96-well Inmulon 2 plates with rabbit anti-human butyrylcholinesterase (Dako #A0032) at 10 mg/ml in PBS (~~100 ml/well~~ **100 µL/well**) overnight at 4°C.

2) Remove coating solution and block plate with 3% BSA in PBS (~~250 ml/well~~ **250 µL/well**) for 2 hours at room temperature.

3) Add ~~200 ml~~ **200 µL** butyrylcholinesterase variant conditioned media and incubate at RT for 2 hrs.

4) Wash plate 3 times with ~~250 ml/well~~ **250 µL/well** PBS.

5) Add ~~85 ml/well~~ **85 µL/well** 0.1 M potassium phosphate pH 7.0

6) Add 13.6 mg o-NPA to 100 ml acetonitrile, mix to dissolve. Add 100 ml of this stock to 6.3 mls water and mix well.

7) Wash plate 3 times with PBS.

8) Add ~~15 ml~~ **15 µL** of diluted o-NPA substrate.

9) Read absorbance at 405 nm.

• At page 67, line 26 through page 68, line 10, insert the following replacement paragraph:

Butyrylcholinesterase variants were assayed for CPT-11 conversion activity using fluorescent High Performance Liquid ~~Chromotography~~ **Chromatography** (HPLC) detection of SN-38 formation as described in Dodds and Rivory, Mol. Pharmacol. 56:1346-1353 (1999), which is incorporated herein by reference. The conversion of CPT-11 to SN-38 is shown in Figure 2. Briefly, conditioned media from transiently expressed BChE variants were exposed to 20 mM CPT-11 for 72 hours at 37°C and analyzed by HPLC for SN-38 formation (peak at about 4 minutes column retention time). Figure 3 shows the amount of SN-38 produced using conditioned media from cells that were mock-transfected which means that the transfection was performed as usual however no DNA was added. Figure 4 shows the amount of SN-38 produced using conditioned media from cells that were transfected with F227A, and figure 5 shows the amount of SN-38 produced using conditioned media from cells that were transfected with F227A/L286S.

• At page 69, lines 8-9, insert the following replacement paragraph:

Butyrylcholinesterase-Mediated Cytotoxicity and ~~Enchanced~~ **Enhanced** CPT-11 Activated Killing by Butyrylcholinesterase Variants

• At page 69, line 12 through page 70, line 7, insert the following replacement paragraph:

A cellular cytotoxicity assay was used to demonstrate the level of CPT-11 activation by BChE variants. Clinically relevant concentrations of CPT-11 (0.5-10 mM) were exposed to BChE variants for 24-72 hours at 37°C. Briefly, CPT-11 at 4 mM was incubated for 72 hours with expressed wild-type BChE, the 6-6 variant, or F227A/L286Q variant. SW48 colon carcinoma cells were exposed to the activated CPT-11 at a concentration of 0.5 mM for 72 hours and cell viability measured by the MTT method. The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay is commercially available and can be used to measure cell viability based on the ability of a cell to reduce a redox sensitive dye. Note the 6-6 variant is a quadruple mutant referenced as A328W/Y332M/S287G/F227A (SEQ ID NO: 16) in the nomenclature used throughout to describe variants. The 6-6 variant contains the following codons at variant positions: GCG encodes alanine at amino acid position 227, GGT encodes glycine at amino acid position 287, ATG encodes methionine at

amino acid position 332, and TGG encodes ~~tryptophane~~ **tryptophan** at amino acid position 328.

• At page 71, line 11 through page 72, line 14, insert the following three replacement paragraphs:

The protocol for anti-Kappa capture of anti-EGFR-L530 is as follows:

1) Coat 96-well Immulon 2 plates with 200 ml of 10 mg/ml anti-human Kappa antibody in PBS overnight.

2) Block plate with 3% BSA in PBS (~~250 ml/well~~ **250 µL/well**) for 2 hours at room temperature.

3) Wash plate 3x with ~~250 ml/well~~ **250 µL/well** PBS.

4) Add ~~200 ml~~ **200 µL** BChE conditioned media and incubate at RT for 2 hours.

5) Prepare working solution of DTNB by making a 1:10 dilution of stock 5 mM DTNB in 0.1 M Potassium phosphate pH 7.0. Add ~~180 ml~~ **180 µL** per well.

6) Prepare working solution of ~~Butyrythiocholine~~ **Butyrylthiocholine** (BTC) by making 1:20 dilution of 200 mM stock in water. Add ~~20 ml~~ **20 µL** per well.

7) Incubate plate at 37°C and read on a spectrophotometer at A405 nm.

An ELISA assay measuring butyrylcholinesterase activity of the anti-EGFR- BChE L530 specifically bound to a cell membrane preparation containing the EGFR antigen is shown in Figure 8. These ~~results demonstrates~~ **results demonstrate** antigen-specific binding of the fusion protein through the antibody domain and enzymatic activity of the butyrylcholinesterase domain.

The protocol for anti-EGFR binding to A431 membrane preparations is as follows:

1) Coat 96-well Immulon 2 plates with ~~50 ml/well~~ **50 µL/well** of A431 cell lysate diluted 1/20 in 10mM HEPES pH 7.4, 0.1% Triton X-100 and dry in the hood overnight.

2) Block plate with 3% BSA in PBS (~~250 ml/well~~ **250 µL/well**) for 2 hours at room temperature.

3) Wash plate 3x with ~~250 ml/well~~ **250 µL/well** PBS.

4) Add ~~200 ml~~ **200 µL** BChE conditioned media and incubate at RT for 2 hours.

5) Prepare working solution of DTNB by making a 1:10 dilution of stock 5 mM DTNB in 0.1 M Potassium phosphate pH 7.0. Add ~~180 ml~~ **180 µL** per well.

6) Prepare working solution of ~~Butyrythiocholine~~ **Butyrylthiocholine** (BTC) by making 1:20 dilution of 200 mM stock in water. Add ~~20 ml~~ **20 µL** per well.

7) Incubate plate at 37°C and read on a spectrophotometer at A405 nm.

• At page 72, line 20 through page 73, line 24, insert the following two replacement paragraphs:

To purify the butyrylcholinesterase variants, the culture medium corresponding to each variant is filtered through Whatman #1 filter paper (Whatman Inc., Clifton, NJ) on a Buchner funnel. The filtrate is poured through a chromatography column (XK50/30, Pharmacia Biotech, ~~Piscataway~~ **Piscataway**, NJ) packed with 100ml of affinity gel procainamide-Sepharose 4B. The butyrylcholinesterase variants stick to the affinity gel during loading so that 20 mg of enzyme that was previously in liters is concentrated in 100ml of affinity gel. The affinity gel is subsequently washed with .3M sodium chloride in 20mM potassium phosphate pH 7.0 and 1mM EDTA to elute contaminating proteins. Next, the affinity gel is washed with buffer containing 20mM potassium phosphate and 1 mM EDTA pH 7.0 to reduce the ionic strength. Finally, the ~~butyrylcholinesterasae~~ **butyrylcholinesterase** ~~variants is~~ **variants are** eluted with 250 ml of 0.2M procainamide in buffer.

To further purify the butyrylcholinesterase variants and remove the procainamide a second purification step can be performed. The butyrylcholinesterase variants recovered in the first purification step are diluted 10-fold with buffer (20 mM TrisCl, 1 mM EDTA pH 7.4) to reduce the ionic strength to about 0.02M. The diluted enzyme is loaded onto a column containing 400ml of the weak anion exchanger DE52 (Whatman, Clifton, NJ). At this low ionic strength the butyrylcholinesterase variant sticks to the ion exchange gel. After loading is complete the column is washed with 2 liters of buffer containing 20mM TrisCl and 1mM EDTA pH 7.4 until the absorbency of the eluant at 280nm is nearly zero, indicating that the procainamide has washed off. Subsequently, the butyrylcholinesterase variants are eluted from the column with a salt gradient from 0 to 0.2M NaCl in 20mM TrisCl pH 7.4.

Following the elution of the butyrylcholinesterase variants 10ml fractions are collected for each variant using a fraction collector. Activity assays are performed to identify the peak containing butyrylcholinesterase variant. SDS gel electrophoresis can be performed to determine the purity of each butyrylcholinesterase ~~variants~~ **variant**, which is typically determined to be approximately 90%.

• At page 74, lines 4-20, insert the following replacement paragraph:

CD20 is a useful target antigen to test the feasibility of using optimized BChE in ADEPT as the antigen is abundantly expressed on human B lymphoma lines (3.2×10^5 molecules/cell) and does not undergo significant internalization upon antibody binding. AME133 is a humanized anti-CD20 with fully human germline framework regions shown in ~~Figures 19 and 20~~ **Figure 20** and set forth as SEQ ID ~~NOS 198 and 200~~ **NO: 198**, with corresponding nucleic acid sequences SEQ ID ~~NOS 197 and 199~~ **NO: 197**. This antibody was generated at Applied Molecular Evolution using the company's directed evolution strategies, and the monovalent Fab binds to CD20 with extremely high affinity (1×10^{-9} M). An exemplary model fusion protein (anti-CD20-BChE.4-1) was generated and is shown in Figure 19. Anti-CD20-BChE.4-1 (SEQ ID NO: ~~202~~ **200**) is composed of AME133 Fab fused at the C-terminal end of the CH1 heavy chain domain to the N-terminus of modified BChE variant L530 (SEQ ID NO: ~~204~~ **201**), which is a functional fragment of reference SEQ ID NO: 180. The BChE variant was truncated at amino acid 530 to abrogate its normal assembly into tetramers. The monomeric version of the enzyme exhibits the equivalent activity of each subunit of the naturally occurring tetrameric form, with no loss of activity due to allosteric effects as described by Blong et al., Biochem J 327 (Pt 3): 747-57 (1997).

• At page 75, line 23 through page 76, line 2, insert the following replacement paragraph:

In patients treated with 200 ~~mg/m2~~ **mg/m^2** CPT-11, plasma concentrations of CPT-11 remained above 0.1 μM for at least 24 hours (Ducreux et al., Ann Oncol 14 Suppl 2: 1117-23 (2003)). CPT-11 is dosed as a single agent at up to 500 mg/m^2 and plasma peak concentration is dose-proportional (Mathijssen et al. Clin Cancer Res 7: 2182-94 (2001); Ducreux et al., *supra,* 2003). These findings demonstrate the targeted killing of tumor cells

with anti-CD20-BChE.4-1 in vitro at concentrations of CPT-11 that are pharmacologically relevant and sustainable over time in patients.

- At page 76, lines 5-10, insert the following replacement paragraph:

This example demonstrates ~~identifation~~ **identification** of additive variants incorporating beneficial mutations from different library regions that resulted, *inter alia,* in the isolation of the H77F, F227A, P285N, V331A variant (SEQ ID NO: 180), also referred to as the 4-1 variant, exhibiting a >3000-fold increase in CPT-11 hydrolysis over wild-type BChE (Figure 12) and a 5-6-fold improvement in the Km for CPT-11, from 40 μM to ~7 μM.